Cardiovascular Regenerative Technologies: Update and Future Outlook

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Abstract: In the effort of improving treatment for cardiovascular disease (CVD), scientists struggle with the lack of the regenerative capacities of finally differentiated cardiovascular tissues. In this context, the advancements in regenerative medicine contributed to the development of cell-based therapies as well as macro- and micro-scale tissue-engineering technologies. The current experimental approaches focus on different regenerative strategies including a broad spectrum of techniques such as paracrine-based stimulation of autologous cardiac stem cells, mesenchymal cell injections, 3D microtissue culture techniques and vascular tissue-engineering methods. These potential next-generation strategies are leading the way to a revolution in addressing CVD, and numerous studies are now undertaken to assess their therapeutic value. With this review, we provide an update on the current research directions, on their major challenges, limitations, and achievements.

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Cardiovascular Regenerative Technologies: Update and Future Outlook

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Keywords
Regenerative medicine · Cell therapy · Tissue engineering · Cardiovascular disease · Ischemic heart disease · Heart valve disease

Summary
In the effort of improving treatment for cardiovascular disease (CVD), scientists struggle with the lack of the regenerative capacities of finally differentiated cardiovascular tissues. In this context, the advancements in regenerative medicine contributed to the development of cell-based therapies as well as macro- and micro-scale tissue-engineering technologies. The current experimental approaches focus on different regenerative strategies including a broad spectrum of techniques such as paracrine-based stimulation of autologous cardiac stem cells, mesenchymal cell injections, 3D microtissue culture techniques and vascular tissue-engineering methods. These potential next-generation strategies are leading the way to a revolution in addressing CVD, and numerous studies are now undertaken to assess their therapeutic value. With this review, we provide an update on the current research directions, on their major challenges, limitations, and achievements.

Introduction

Cardiovascular disease (CVD) includes a plethora of disorders affecting the cardiovascular system comprising, among others, heart failure (HF) and heart valve disease (HVD). Frequently, HF is caused by coronary artery occlusion and can lead to acute ischemic episodes (ischemic heart disease; IHD). Due to poor or absent post-ischemic reperfusion of the infarcted myocardial areas, a significant amount of cardiomyocytes undergoes apoptosis and is replaced by fibrous scar tissue. The relative unpredictability of acute cardiovascular events, the high mortality rate and the absence of a durable post-ischemic treatment, endorse IHD to one of the most critical clinical hurdle of this century. The long-term survival of patients with heart failure is compromised by a series of complications as the massive proliferation of non-contractile fibrotic tissue in the infarcted areas. Additionally, after acute ischemic episodes, patients endure life-changing treatments ranging from daily medications to surgical interventions, e.g., pacemakers, stents, angioplasty or heart transplants. In this context, regenerative medicine can be considered as innovative and valid alternative. The identification of progenitor cardiac cells within the adult human heart [1] encouraged the enthusiasm for cell-based regenerative therapies [2–4]. Moreover, the evidence that exogenous cells injected in the myocardium could minimally promote the formation of functional contractile tissue in the scarred areas provided an important contribution in directing the preclinical research towards the investigation of possible new treatments [5].Together with IHD, HVD represents a leading cause of mortality worldwide [6]. Different valve pathologies are grouped under the HVD acronym, e.g., valve stenosis or prolapse; the latter are mainly caused by congenital heart valve defects, infections or age-related pathological changes. New techniques and prostheses for valve replacement have been introduced into clinical routine, increasing the range of treatment opportunities for the patients. Despite these efforts, today's heart valve artificial prostheses are still associated with increasing risk of thrombogenic events, progressive degeneration, and calcification. In this scenario, regenerative medicine technologies such as tissue engineering hold the potential to overcome the wide range of harmful limitations. The concept of heart valve tissue engineering encompasses the isolation, expansion, and seeding of patient-derived cells onto biodegradable scaffolds; this process is followed by in vitro culture and subsequent in vivo implantation of the final construct into the patient. The resulting bioengineered valve can potentially provide non-immunogenic, non-thrombogenic characteristics together with growth and remodeling properties. The research in the

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field is now focusing on new cell sources and new biomaterials; the optimal combination of these two factors could indeed contribute in raising the versatility and extending the horizons of the field of heart valve tissue engineering.

Regenerative Technologies for Heart Failure

Despite the recent advances of basic research in the field, the detailed architecture of cardiac repair response upon ischemia is currently not clear. The presence of progenitor cells capable of a local and limited regenerative activity in the adult heart inspired the study and development of both i) cell-based technologies and ii) strategies for paracrine stimulation of the resident cardiac progenitor cells. The next paragraphs will introduce and discuss both regenerative approaches.

Cell-Based Regenerative Technologies: From Single Cell to Bioengineered Microtissues

Over the past decades preclinical and clinical investigations of cell-based technologies for the treatment of the infarcted heart have been carried out. Different cell candidates were evaluated in vitro and are now under investigation for a possible therapeutic use. Examples are autologous in vitro-expanded cardiac progenitor cells (CPCs) and autologous adipose tissue- or bone marrow-derived mesenchymal stem cells (MSCs) [7]. Although clinical trials based on the intra-myocardial injection of single-cell suspensions have been performed with mesenchymal autologous cells, the interpretation of the results is controversial and roused questions and doubts regarding the power of the technology [8–10]. There is currently a consensus regarding the possible reasons for the single-cell suspension therapy failure; the negative results are addressed to the scarce survival and retention of the cells at the level of the infarcted area [11]. The newest regenerative approaches aim at enhancing survival, grafting, and retention abilities of the injected cells using different methodologies. An example is provided by the cutting-edge bioengineered microtissue technology. Microtissues are scaffold-free tissue-engineered spheroids generated via hanging drop technique. These microstructures are reproducible with different cell types like CPCs, bone marrow- and adipose tissue-derived MSCs [4] and have been tested in a pilot study on infarcted porcine models exhibiting promising results [12]. Other methods currently investigated for increasing cell retention in the infarcted areas include i) the combination of single cell suspensions with nanoparticles loaded with functional agents [13], ii) the injection of a compact mixture of cells pre-cultured in hydrogels [14], or iii) the injection of cell-free hydrogels functionalized with paracrine mediators [15].

Paracrine Stimulation of Resident Progenitor Cells

The self-regenerative ability of the adult heart is per se inefficient to stimulate the functional replacement of necrotic areas and prevent the formation of fibrous tissue after myocardial infarction. Regenerative mechanisms demand tailored triggers that are provided by cell-cell communication. It is known that epicardium and endocardium share a communication network based on vesicular exchange [16]; cells can indeed exchange information by packing and delivering ribonucleic acids and proteins within microvesicles known as exosomes (EVs) [17–19]. A branch of recently developed regenerative technologies adopted this mechanism of cell-cell communication to stimulate and increase the physiological regenerative processes upon injury. The vesicular content of EVs varies according to environmental clues (i.e., hypoxic conditions or acute myocardial infarction) and provides growth factors, anti-apoptotic and angiogenic as well as mitogenic signals. In detail, EVs are predominantly enriched in microRNAs (miRNA), small non-coding RNA molecules with the function of post-transcriptional regulators [20]. Cardiomyocyte-specific extracellular factors and miRNAs (e.g. miR-1, miR-133, and miR-206) are key regulators of cardiac function and are released in high concentrations upon heart injury, exerting their regulatory role on the surrounding cells [21, 22]. The beneficial and synergic function of specific combinations of miRNAs on CPCs has been proven together with the hypothesis that miRNA families can selectively be secreted into the extracellular environment via exosomes [23, 24]. The recent EV-inspired regenerative technologies focus either on i) the drug-exerted stimulation of cardiac progenitors [25, 26] or ii) the in situ miRNA-targeted delivery [27]. Different studies contributed to the development of these technologies, like the in vitro and in vivo investigation of the secretome and extracellular vesicular content of MSCs. These studies led to the discovery of new cardioprotective molecules [28–31]. In this regard, Timmers et al. [30] showed in 2007 the beneficial effects of MSC-conditioned medium upon injection in a pig model of ischemia/reperfusion, reporting a significant reduction in the ischemic myocardial area after the treatment. More recently, the secretome of human amniotic membrane-derived MSCs was injected in infarcted rat models, leading to a reduction of the ischemic area and ventricular remodeling [28]. Although these promising studies are still at the preclinical level, they have the potential to shorten the distance from clinical trials to a possible therapeutic approach in humans [32].

Regenerative Technologies for Heart Valve Disease

The landscape of medical treatments for valvular disease is in constant expansion, providing hope in the possibility of improving HVD patients' life expectancies. The most investigated and performed treatment for late-stage valvular dysfunctions is, at the moment, heart valve replacement [6]. Different types of for valvular substitutes are available but the best option must be chosen considering different key parameters such as thromboresistance, implantability, hemodynamics, and durability of the substitute device. The constant search for a durable, safe alternative for heart valve replacement stimulated a rapid development in the field of tissue engineering. The bioengineered constructs are indeed autologous and potentially able to grow and adapt to the changing organ anatomy of a child, avoiding the hurdle of iterated surgery (fig. 1) [33, 34].

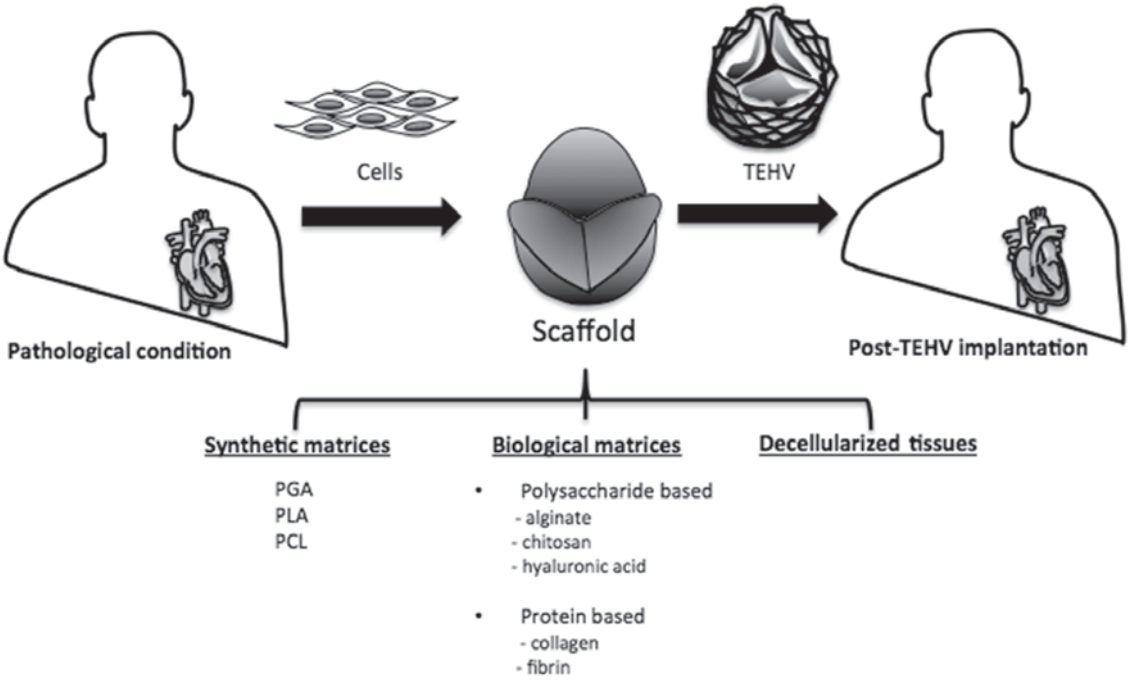

Fig. 1. Overview of the tissue engineering approach and scaffold matrices. Cells are seeded on support matrix shaped like a trileaflet heart valve. In the figure peculiar scaffold materials for tissue engineering are summarized and include i) synthetic matrices, ii) biological matrices, and iii) decellularized tissues. The final goal is the production of a tissue engineered heart valve (TEHV) characterized by optimal durability, thromboresistance, and hemodynamic profile.

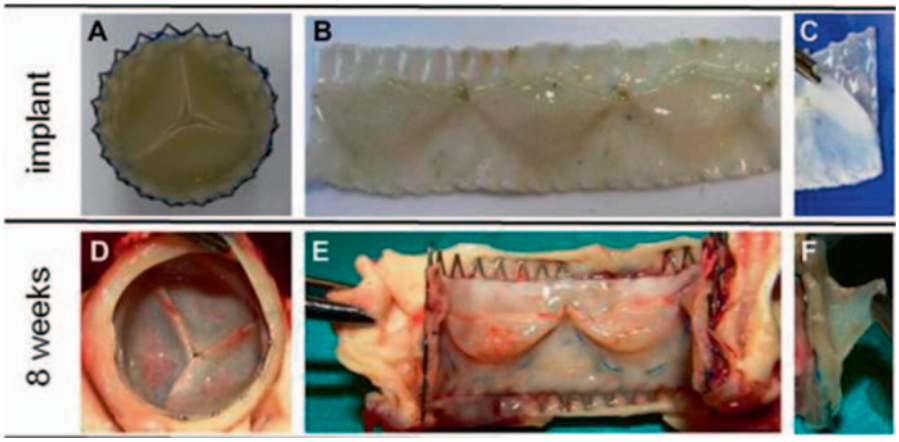

Fig. 2. Macroscopic appearance of decellularized in vitro grown tissue engineered heart valve. Adapted from Driessen et al. 2014 [34]. Macroscopic appearance of an implanted (**A–C**) and explanted (**D–F**) decellularized in vitro grown heart valve in closed configuration (**A, D**), opened configuration (**B, E**), and as cross section (**C, F**).

Tissue Engineering Technologies for Heart Valve Replacement

Tissue engineering approaches are largely investigated in the field cardiovascular medicine and mainly rely on the fabrication of tissue-engineered blood vessels and heart valves. The key steps in today's heart valve in vitro fabrication start with the isolation of cells from the patient. Cells are then expanded, characterized, and seeded onto biodegradable molds (fig. 1). Due to extracellular matrix (ECM) production and concomitant scaffold degradation, the bioengineered heart valve is shaped and in vitro manufactured; potentially ready to be implanted [35–37]. Advanced tissue engineering approaches are based on dynamic, pulsatile flow culturing strategies. These are fulfilled using in vitro bioreactor systems to simulate physiological mechanical stimuli provided by blood pressure and flow [38]. Recently, a second, promising regenerative technology, known as in situ tissue engineering, has been investigated. The latter is based on the hypothesis that the most appropriate scaffold for tissue engineering is the ECM itself. According to this hypothesis, spontaneous scaffold-driven endogenous cell recruitment and migration may occur in situ, leading to a complete ECM scaffold repopulation. To achieve this goal, native matrices are obtained through tissue decellularization and tested for in situ tissue engineering (fig. 2) [34, 39–41].

Biomaterials for Heart Valve Tissue Engineering

The chemical composition of the scaffold matrices employed for tissue engineering is crucial for the success of the valvular fabrication process. The predominant scaffolding approach is based on the use of pore networks of biodegradable scaffolds [37, 42]. These porous networks are important for nutrient supply and are the common standard for the design of new biomaterials because they guarantee high reproducibility and low costs as well as remarkable mechanical strength and predictable physical properties. Many studies have investigated various fabrication techniques in order to generate novel structures for tissue engineering applications, e.g. polymers assembled with various electrospun designs [43–46]. Today's attempts in developing scaffolds for tissue engineering are

proceeding in two directions and are based on the use of more or less complex polymers from different sources that are either i) fully synthetic or based on ii) biological matrices. A huge variety of biodegradable synthetic polymers have been synthesized and historically proposed as suitable tissue engineering matrices [47], for example, polyglycolic acid (PGA), polylactic acid (PLA), poly-4-hydroxybutyrate (P4HB) and polycaprolactone (PCL) all suitable for tissue engineering, being biodegradable and varying in manufacturing shapes (fig. 1). Among these, aliphatic polyester composite structures are considered as one of the best choices for tissue engineering. One of their main features of interest resides in their relative resistance to fast hydrolytic degradation, which is a discriminatory point given the obliged culture in aqueous media [48]. Their erosive degradation starts from the ester bonds in their polymeric backbone and is continued by random hydrolysis and concomitant enzymatic esterase activity [49, 50] while the degradation products are bio-resorbed in the common cellular metabolic pathways. The scaffold produced using biological matrices can be used alone or in combination with synthetic polymers [51]. The large class of bioscaffolds includes, among the others, polysaccharides such as alginate and proteins such as collagen and fibrin [52]. These biomaterials were the subject of a recent revolution starting with the advent of 3D-bioprinting techniques. In 2013 Duan et al. [53] performed 3D-bioprint living alginate/gelatin hydrogel aortic valves, incorporating within the biomaterial both smooth muscle cells and aortic valve leaflet interstitial cells, the main cell populations in the valve leaflets. However, despite the initial success of the biomatrices, the synthetic materials are still considered a better choice for tissue engineering due to their high durability. Of great interest among biomaterials used for heart valve tissue engineering are the commercially produced polyhydroxylalkanoates, microbial polyesters that can be manufactured with the desired length and degradation time [54]. This family includes polymers such as poly-4-hydroxybutyrate (P4HB) generally combined with other synthetic polymers (e.g. PGA) for the final manufacture of a hybrid scaffold. PGA-P4HB is the today's most used combination for the production of tri-leaflet heart valves due to the successful coupling of material-related biophysical characteristics, joining the high porosity of PGA with the thermoplasticity of P4HB. The decellularized tissue-derived matrices conclude the excursus on biomaterials for heart valve regenerative technologies. The possibility to use tissue-derived templates made of naturally assembled networks of ECM is inevitably interesting as an alternative to the materials described above. One of the main unsolved questions is the matter of the donor source. The latter has to be properly chosen to avoid a possible immune response in the host or disease transmission [55]. Therefore, the choice of the donor tissue must be accurate; today's most used decellularized scaffold matrices are derived from homografts or xenografts [56]. A recent study investigated the repopulation capacity of decellularized tissue-engineered heart valves in a non-human primate model up to 8 weeks [39], showing a rapid cellular repopulation of the decellularized constructs and high remodeling capacities. This study underlines the strong translational power of this technology.

Cell Sources for Heart Valve Tissue Engineering

Other crucial variables to be considered for a fruitful in vitro tissue-engineered valvular production include i) the cell type used for the seeding onto the valvular molds, ii) the culture type, meaning either single cell type cultures or co-culture systems, and iii) ECM production coupled with biomaterial degradation rates [57]. Additionally, cells should be of autologous origin, non-immunogenic, and possessing pronounced plasticity. A wide panel of cell types with such characteristics has been investigated for tissue engineering, like vascular tissue, adipose tissue, umbilical cord blood, chorionic villi, amniotic fluid-derived cells, or cells directly differentiated from patients' induced pluripotent stem cells (iPSCs). Cells derived from vascular tissues (e.g. from peripheral arteries or umbilical veins) are so far the cell types preferred for scaffold seeding for tissue engineered heart valve production [6]. Usually, two main cell populations are isolated from this tissue type: endothelial cells and myofibroblasts. The latter are responsible for the creation of a stable environment for the endothelial cells by depositing ECM, while endothelial cells generate a tight and confluent layer [33, 40]. Stem cells are one of today's most interesting options for tissue engineered valve production [37] due to their fast growth and extensive potential to differentiate towards different cell fates. Among them, MSCs not only share important phenotypic traits with the valve interstitial cells but also retain a great cellular potency and can be isolated with minimally invasive interventions. This endorses MSCs as one of the most suitable stem cell candidates for valvular bioengineering. In recent years, another interesting cell source has become available. In 2006 Yamanaka et al. [58] managed to reprogram adult fibroblasts to a pluripotent status, obtaining iPSCs. Due to their non-embryonic origin, the use of iPSCs for tissue engineering could potentially overcome all ethical problems, allowing to create a patient-specific valvular construct by differentiating the stem cells generated from autologous skin biopsy [59].

Conclusions

The present review summarizes the most recent regenerative technologies developed for the treatment of CVD. In particular, it provides an overview of the regenerative strategies in the context of IHD and HVD. It is possible to identify few promising concepts that are drawing the future of the field. One of them is the idea of replacing/combining stem cell-based therapies for the infarcted heart with an intra-myocardial injection of a cardioprotective cocktail. Other intriguing concepts are i) the use of microtissues as a possible alternative to cell therapy performed with single-cell suspensions and ii) the heart valve tissue engineering technology. The first is considered a promising cell delivery system for enhancing cellular engraftment and survival in the infarcted myocardium; the second aims at revolutionizing the field of heart valve replacement with the use of decellularized tissues for promoting in situ tissue regeneration.

Disclosure Statement

The authors have not conflicts of interest to report.

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